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Abstract

A direct relationship has been established between synaptic activity and amyloid-β secretion. Dysregulation of neuronal calcium homeostasis was shown to increase production of amyloid-β, contributing to the initiation of Alzheimer's disease. Among the different routes of Ca^{2+} entry, *N*-methyl-D-aspartate (NMDA) receptors, a subtype of ionotropic glutamate receptors, are especially involved in this process because of their ability to gate high levels of Ca^{2+} influx. These receptors have been extensively studied for their crucial roles in synaptic plasticity that underlies learning and memory but also in neurotoxicity occurring during acute brain injuries and neurodegenerative diseases. For one decade, several studies provided evidence that NMDA receptor activation could have distinct consequences on neuronal fate, depending on their location. Synaptic NMDA receptor activation is neuroprotective, whereas extrasynaptic NMDA receptors trigger neuronal death and/or neurodegenerative processes. Recent data suggest that chronic activation of extrasynaptic NMDA receptors leads to a sustained neuronal amyloid-β release and could be involved in the pathogenesis of Alzheimer's disease. Thus, as for other neurological diseases, therapeutic targeting of extrasynaptic NMDA receptors could be a promising strategy. Following this concept, memantine, unlike other NMDA receptor antagonists was shown, to preferentially target the extrasynaptic NMDA receptor signaling pathways, while relatively sparing normal synaptic activity. This molecular mechanism could therefore explain why memantine is, to date, the only clinically approved NMDA receptor antagonist for the treatment of dementia.

Keywords: amyloid-β; amyloid precursor protein; Alzheimer's disease; calcium; memantine; NMDA receptors.

Introduction

Alzheimer's disease (AD) is a chronic disorder that slowly destroys neurons leading to progressive cognitive disability. If the etiology of AD is largely unknown, the neuropathological hallmarks of the disease are well characterized. They include an extracellular deposition of diffuse and neuritic plaques composed of amyloid-β (Aβ) peptide, an intracellular accrual of neurofibrillary tangles of hyperphosphorylated microtubule-associated protein TAU, synaptic dysfunctions and a selective neuronal loss (Selkoe, 2001). These histopathological lesions do not occur diffusely throughout the brain parenchyma but are particularly restricted to the cortex, hippocampus and amygdale (Selkoe and Shenk, 2003). Although neurofibrillary tangles are commonly found in diverse neurodegenerative diseases, amyloid plaques are only a hallmark of AD. Thus, the leading candidate for the trigger of AD is supposed to be the Aβ peptide which is produced by the proteolytic processing of the amyloid precursor protein (APP) (Hardy and Selkoe, 2002). Its primacy has been manifested in the well known 'amyloid-cascade hypothesis' (Citron, 2004). This hypothesis suggested that the mismetabolism of APP was the initiating event in AD pathogenesis, subsequently leading to disruption of synaptic connections as well as the aggregation of Aβ, specifically Aβ42 (Hardy and Allsop, 1991; Tanzi and Bertram, 2005). Among the different causes proposed to explain the increased production of Aβ, dysregulation of neuronal calcium homeostasis was first postulated more than 20 years ago (Khachaturian, 1987, 1989, 1994). This theory was supported by experimental studies showing alterations in calcium signaling both in sporadic and in familial cases of AD (Mattson, 2004; Smith et al., 2005; Stutzmann, 2005). Comparatively to other routes of Ca^{2+} entry in neuronal cells, *N*-methyl-D-aspartate receptors (NMDARs), a subtype of ionotropic glutamate receptors, are of particular interest because of their special ability to gate high levels of Ca^{2+} influx (Choi et al., 1988; Sattler et al., 1998). These receptors have been extensively studied for their crucial role in synaptic plasticity and excitotoxicity (Rothman and Olney, 1995; Waxman and Lynch, 2005). Moreover, excessive NMDAR activity was shown to contribute importantly to the etiology of many acute or chronic neurodegenerative disorders such as stroke, Hungtinton's disease, HIV-associated dementia and AD (Lipton and Rosenberg, 1994; Lancelot and Beal, 1998). Several studies reported a direct link between NMDAR overactivation on the one hand, and APP processing and neuronal Aβ secretion on the other hand, even if the conclusions could be somewhat different (Lesné et al., 2005; Marcello et al., 2007; Hoey et al., 2009). It was further demonstrated that the

cellular location of NMDARs is a key parameter controlling their effect on neuronal viability. While activation of synaptic NMDARs was shown to drive neuroprotective gene transcription and to promote neuron survival, stimulation of extrasynaptic NMDARs triggers neuronal death (Hardingham et al., 2002; Léveillé et al., 2008). In the same way, this dichotomous nature of NMDAR signaling has also been outlined in the field of neurological disorders (Hardingham and Bading, 2010). Recently, our group provided data suggesting that perturbations in the balance between synaptic and extrasynaptic NMDAR activity could increase neuronal Aβ production, contributing to the pathogenesis of AD (Bordji et al., 2010).

The present review focuses on the relationship between NMDAR activation and neuronal Aβ production. We outline the role of this subtype of glutamate receptors in calcium dysregulation which seems to be central to the pathogenesis of AD. Finally, from the available data, we examine prospects for therapies using memantine, the only clinically approved NMDAR antagonist for the treatment of AD.

APP metabolism and Aβ formation

Around 25 years ago, the Aβ peptide was first recognized to be derived from its large APP, by sequential endoproteolytic cleavage (Kang et al., 1987). APP is a type-1 membrane glycoprotein found at the cell surface, in endosomal, endoplasmic reticulum and Golgi membranes in many cell types. The protein is encoded by the *APP* gene located on chromosome 21. Individuals with Down's syndrome, who have three copies of chromosome 21, and hence three copies of the *APP* gene, develop clinical and pathological signs of early-onset Alzheimer's. Several possible neuronal functions have been ascribed to APP holoprotein and/or their secreted soluble derivatives (sAPPα) (Qiu et al., 1995). sAPPα acts as an autocrine factor (Saitoh et al., 1989) and can exhibit some neuroprotective or neurotrophic properties (Mattson et al., 1993). The function of APP is unclear and deletion of the *APP* gene in mice results in neither early mortality nor appreciable morbidity. This absence of vital consequence of APP deletion could be explained by the expression of closely related proteins, the amyloid precursor-like proteins (APLP1 and APLP2). More recent *in vitro* studies reported that APP might be an axonal transport receptor and might function in cellular interactions when inserted at the plasma membrane (Kamal et al., 2000; Stokin and Goldstein, 2006). The protein was also suggested to be involved in the activation of G-protein-dependent pathway (Nishimoto et al., 1993), leading to apoptosis activation (Mbebi et al., 2002). Moreover, the cytoplasmic carboxyl-terminal domain of APP has been shown to be transported to the nucleus to modulate calcium signaling, underlying its role in signal transduction (Cao and Sudhof, 2001; Leissring et al., 2002).

The processing of APP takes place according to the general pattern of regulated intramembrane proteolysis. It undergoes two consecutive endoproteolytic cleavages that either preclude or cause the formation of the amyloidogenic Aβ peptide (Selkoe, 2001). In a first step, the extracellular part of APP is cleaved from the cell surface, either by α- or β-secretase. Three members of the ADAM family (a disintegrin- and metalloproteinase family enzyme) ADAM9, ADAM10 and ADAM17 have been shown to display α-secretase activity (Lammich et al., 1999). The prevalent cutting of APP by α-secretase precludes Aβ formation as the cleavage site is localized inside the amyloid sequence between residues 16 and 17. This enzymatic action leads to the release of a large soluble extracellular amino terminal fragment of APP (sAPPα) and of a non-amyloidogenic 83-amino acid C-terminal fragment (C83). Conversely, in an amyloidogenic pathway, APP could be alternatively cleaved at a position located 99 amino acids from the C-terminus by a β-secretase, an aspartyl protease known as BACE1 (β-site APP-cleaving enzyme) (Hussain et al., 1999). Cleavage by β-secretase releases a slightly shorter soluble APP peptide (sAPPβ) in the extracellular space and leaves a 99-amino acids C-terminal fragment (C99) within the membrane. In a second step of APP proteolysis, C83 or C99 are cleaved by γ-secretase within the lipid bilayer to generate, respectively, the release of the non-amyloidogenic p3 peptide or of the 40–42 amino acid residues long Aβ. This intramembrane cleavage is a rather unusual cellular process and it was initially found to be impossible at a biochemical level (Steiner and Haass, 2000). The γ-secretase has been identified to be a high-molecular weight active complex composed of presenilin 1 or 2 (PS1 and PS2), nicastrin, anterior pharynx-defective phenotype (aph-1) and PS-enhancer (PEN-2) (Francis et al., 2002). The $A\beta_{1-42}$ variant exhibits higher hydrophobic properties than $A\beta_{1-40}$ and thus is more susceptible to be involved in fibril formation. Indeed, the longer isoform is primarily found in the diffuse and neuritic plaques. Conversely, $A\beta_{1-42}$ represents only approximately 10% of the total Aβ species produced in the brain parenchyma, whereas $A\beta_{1-40}$ is the more abundant isoform released following APP processing (Haass, 2004).

It was shown that Aβ production was a normal cellular process because it was detected in cerebrospinal fluid and plasma of healthy subjects throughout life. One pivotal question, still open, is to know at which state Aβ peptides begin to become pathological. Accumulation of Aβ in cerebral tissues of AD patients could be the result of an impaired clearance of the peptide and/or its overproduction through an altered processing of APP. Thus, all phenomena that cause perturbations of APP processing, linked or not to calcium influx, could modify Aβ generation and play a central role in AD pathogenesis.

Neuronal activity, APP metabolism and Aβ production

For many years, AD was attributed to the deposit of fibrillar Aβ which causes the generation of neurofibrillar tangles and ultimately the death of neurons. In a more recent version of the amyloid cascade hypothesis, it is proposed that soluble Aβ oligomers are responsible for the early cognitive decline in AD (Walsh and Selkoe, 2007). Thus, extensive studies have shown that these oligomers rapidly disrupt synaptic

functions progressively leading to synaptic loss and neuronal death (Shankar et al., 2007). In the same way, they were also described to inhibit long-term potentiation and to facilitate long-term depression (Li et al., 2009). There is now ample evidence that Aβ oligomers do not affect neuronal viability directly but interfere specifically with synaptic function. In patients suffering from AD, memory impairment or loss strongly correlates with cortical levels of soluble Aβ species, especially oligomers.

If the role of APP in calcium signaling and the effects of Aβ on synaptic dysfunction and loss have been thoroughly documented, there are much less data on how neuronal activity could modulate neuronal APP metabolism and transport, and extracellular Aβ release. The first study to show that Aβ formation can be increased by calcium influx through calcium channels of the plasma membrane, or calcium release from endoplasmic reticulum stores was by Querfurth and Selkoe (1994). Then, several other reports provided circumstantial evidence suggesting that, in both animal models and humans, Aβ levels could be related to synaptic activity. First, using positron emission tomography and functional magnetic resonance imaging, it was evidenced that the highest Aβ accumulation and aggregation occurs in brain regions exhibiting elevated metabolic activity at rest, such as hippocampus and parts of frontal and parietal cortex (Buckner et al., 2005). High metabolic activity is generally well correlated to high neuronal activities. Second, a relationship has been reported between stress, neuronal activity and AD. Patients that are prone to psychological distress are more likely to develop AD. A significantly higher Aβ level was also measured in the interstitial fluid (ISF) of APP transgenic mice subjected to restraint stress when compared to the corresponding animal controls (Palop et al., 2007). Finally, some patients suffering from temporal lobe epilepsy, thus exhibiting substantially elevated neuronal activity, were shown to develop diffuse Aβ deposits in these regions of the brain very early in life (Mackenzie and Miller, 1994).

More recently, different investigators interestingly reported that synaptic activity modulates Aβ metabolism. Kamenetz and coworkers demonstrated that increased synaptic activity enhanced the formation and secretion of Aβ peptide in hippocampal slice neurons (Kamenetz et al., 2003). In turn, Aβ selectively depresses excitatory synaptic transmission onto neurons that overexpress APP as well as nearby neurons that do not. This depression can be reversed by blockade of neuronal activity, especially using NMDAR antagonists, demonstrating the involvement of this subtype of receptor. Additionally, they propose that activity-dependent modulation of endogenous Aβ production could normally participate in a feedback that could keep neuronal hyperactivity in check. Disruption of this feedback system could contribute to disease progression in AD.

In another *in vivo* study, Cirrito and coworkers used a microdialysis technique in tandem with concurrent hippocampal electrophysiological recording to assess whether there are dynamic changes in ISF Aβ levels in conjunction with different levels of neuronal activity in awake, behaving mice (Cirrito et al., 2005). They demonstrated that Aβ levels in the brain ISF are dynamically and directly influenced by synaptic activity on a timescale of minutes to hours. When synaptic activity within the hippocampus was increased by electrically stimulating the perforant pathway, Aβ in ISF increased by 30% within 1 h. Conversely, administration of the sodium channel blocker tetrodotoxin, which causes a cessation of neuronal activity, significantly decreased basal electrophysiological activity and subsequently dropped Aβ levels in ISF by 40%. This effect was shown to be reversible, demonstrating a direct relationship between neuronal activity and Aβ concentration in ISF.

In a more recent study performed by the same group, evidence was provided showing that synaptic activity causes an increase in vesicle endocytosis, driving more APP in the endocytotic compartment, ultimately resulting in increased neuronal Aβ production and release (Cirrito et al., 2008).

Because extracellular Aβ is known to reduce synaptic plasticity and alter synaptic function, then increased secretion of Aβ induced by synaptic activity could lead to damage and loss of synapses and to progressive accumulation of extracellular Aβ into amyloid plaques, which are important hallmarks of AD. The consequence of this point of view is that synaptic activity is paradoxically detrimental and could contribute to AD pathogenesis. This seems to be in contradiction with another set of studies supporting the concept that cognitive activity could be protective against AD. Higher educational level or involvement in mentally stimulating activities correlated with a lower probability of developing AD (Stern, 2006). Behavior studies performed with AD transgenic mice demonstrated that environmental enrichment reduced Aβ levels and amyloid deposition (Lazarov et al., 2005).

In fact, the results of these different studies are conflicting in appearance. In both *in vitro* and *in vivo* experiments with activity-induced secretion, the concentration of extracellular Aβ remains in the picomolar range. This concentration range of Aβ was also measured in the ISF of mice subjected to a circadian rhythm, with more elevated levels when awake (Kang et al., 2009). Moreover, other authors reported that Aβ levels in the picomolar range enhanced synaptic plasticity and memory (Puzzo et al., 2008; Garcia-Osta and Alberini, 2009). Thus, these data support the idea that Aβ secretion could be a physiological event occurring with normal brain activity.

Tampellini et al. recently underlined the important role of intraneuronal Aβ in the pathogenesis of AD (Tampellini and Gouras, 2010). They showed that KCl-induced synaptic activity reduced intraneuronal Aβ and protects against Aβ-related synaptic alterations. Neprilysin is involved in the intracellular Aβ decrease and, by live cell imaging, they evidence the transport of APP to synapses promoted by synaptic activity (Tampellini et al., 2009).

Taken together, these reports demonstrate a lack of consensus concerning the effect of neuronal activity (electrical, synaptic or channel-dependent) on Aβ synthesis and release. Important parameters seem to be intracytosolic calcium levels and the method of calcium entry in neurons.

NMDAR activation and neuronal Aβ synthesis

Numerous studies demonstrated that excessive Ca^{2+} entry leading to neuronal Ca^{2+} overload is a key early step in glutamate-induced cell death in excitotoxic processes (Choi, 1995). Among the different routes of calcium entry, NMDARs, a subtype of ionotropic glutamate receptors, are of particular interest because of their special ability to gate high levels of Ca^{2+} influx (Rothman and Olney, 1995). Thus, these receptors have been extensively studied for their crucial role in synaptic plasticity and neuronal damage occurring during acute or chronic pathologies (Waxman and Lynch, 2005). Dennis Choi was the first to demonstrate that the NMDARs were the primary source of toxic Ca^{2+} influx mediating glutamate excitotoxicity (Choi et al., 1988). Shortly afterwards, it was proposed that Ca^{2+} entry through NMDARs was particularly effective at killing neurons as compared with entry through other channels (Tymianski et al., 1993). At the same time, there was circumstantial evidence pointing to a role for glutamate toxicity and NMDAR activity in acute brain injuries (stroke, traumatism) or in chronic neurodegenerative diseases, including Huntington's disease (Choi et al., 1988; Lipton and Rosenberg, 1994; Rothman and Olney, 1995). The involvement of glutamate mediated neurotoxicity in the pathogenesis of AD is now widely accepted. Central to this hypothesis is the assumption that glutamate receptors, especially NMDARs, are continuously overactivated. Such mild and chronic activation leads to impairment of synaptic plasticity (learning) and ultimately to synapse loss and neuronal death.

For several years, we have studied in the lab the link existing between NMDAR activation, APP processing and neuronal Aβ production. We initially demonstrated that a prolonged stimulation of cortical neuron cultures with a sublethal concentration of NMDA increased the production and secretion of Aβ (Lesné et al., 2005). This effect was preceded by an important modification of neuronal APP expression pattern with the expression of an APP isoform which is normally absent in neurons: the Kunitz protease inhibitor (KPI) domain containing APP (KPI-APP). This KPI domain was found to associate with the α-secretase candidate tumor necrosis factor-α converting enzyme (TACE) and to inhibit its enzymatic activity. The consequence of this KPI-dependent inhibition is a shift from α-secretase to β-secretase-mediated APP processing and then to an increase in Aβ production. These results suggested that even mild deregulation of the glutamatergic neurotransmission could increase Aβ production and represent a causal risk for developing AD. It is important to note that the effect reported in this study, with a long-lasting NMDAR activation period, reflected a pathophysiological situation. Indeed, the observed effects (induction of KPI-APP expression followed by Aβ production) were due to transcriptional changes in the APP isoforms expressed in neuronal cultures after prolonged (24 h) NMDAR stimulation.

At another level, some authors have recently investigated whether NMDAR activation could directly modulate APP processing in primary neuron cultures by analyzing the release of soluble APP (sAPP) and APP C-terminal fragments as a reflection of α- and β-secretase activities. They found that NMDAR stimulation, after glutamate or NMDA treatment, increased non-amyloidogenic α-secretase-mediated APP processing (Hoey et al., 2009). This effect was accompanied by a significant decrease in neuronal Aβ release. Importantly, only NMDAR antagonists D-AP5 and MK801 blocked the modifications on APP and Aβ metabolism, in contrast to the AMPA receptor antagonist CNQX or the L-type calcium channel blocker nifedipine. The authors proposed that NMDAR-mediated inhibition of Aβ release is a secondary event resulting from increased α-secretase-mediated cleavage of APP. It has previously been reported that there is a competition between the candidate α-secretases ADAM10 and ADAM17 and β-secretase for APP cleavage (Skovronsky et al., 2000). Thus, an increase in α-secretase-mediated APP cleavage during NMDAR stimulation could limit the amount of APP available for BACE1 cleavage, reducing neuronal Aβ production.

A close relationship between NMDAR stimulation and α-secretase activity has been previously underlined in primary hippocampal neurons. Marcello et al. reported that synapse-associated protein-97, a protein involved in dynamic trafficking of proteins to the excitatory synapse, drives ADAM10 to the postsynaptic membrane in dendritic spines (Marcello et al., 2007). Interestingly, they demonstrated that NMDAR activity directly mediates this event and positively modulates α-secretase activity. Their findings reinforce the concept that the glutamatergic synapse, especially its NMDAR subcomponent, can intrinsically express a mechanism leading to a shift of APP metabolism towards a non-amyloidogenic pathway. It should be noted that other receptor systems have been shown to promote the non-amyloidogenic pathway. In studies performed in animal models or in human, activation of muscarinic M1 acetylcholine receptors has been reported to increase α-secretase cleavage of APP and to decrease Aβ levels in cerebrospinal fluid (Nitsch et al., 2000; Caccamo et al., 2006). The same observations have been made after mGluR1α glutamate receptor activation. Other authors showed that electrical depolarization as well as protein kinase C activation induces sAPPα production in rat hippocampal slices (Caputi et al., 1997).

Whereas the involvement of NMDARs in the excitotoxic process is well established, one should consider that the survival of several types of neurons is strongly dependent on synaptic NMDAR activity and function. Elimination of NMDAR activity *in vivo* causes widespread apoptosis and exacerbates neuron loss when applied after traumatic brain injury or during ongoing neurodegeneration. Thus, although high levels of NMDAR activity can be harmful, so too is blockade of normal synaptic NMDAR activity. This 'NMDAR paradox' explains why the use of NMDAR antagonists is somewhat unsuccessful and has failed in clinical trials.

For several years, it was well accepted that only the degree of Ca^{2+} entry through NMDARs could account for these differences in cell fate: moderate NMDAR activity was considered to be beneficial for neurons, whereas excessive stimulation with subsequent neuronal Ca^{2+} overload was deleterious. This point of view was gradually challenged over the past decade in light of several important papers reporting

that NMDAR activation could have opposite consequences on neuronal viability, according to their cellular localization (synaptic vs. extrasynaptic sites). Stimulation of synaptic NMDARs induces prosurvival events by triggering genomic processes that render neurons more resistant to apoptosis and oxidative insults. Conversely, calcium flux through extrasynaptic NMDARs overrides these pathways, causing mitochondrial dysfunction and cell death (Hardingham et al., 2002). According to this concept, it is not only the level of intracellular calcium concentration that confers neuronal toxicity, but rather the Ca^{2+} influx through NMDARs located outside the synapse. Moreover, several studies have demonstrated that some of the extrasynaptic signaling pathways dominate over effects of synaptic signaling, shutting off some important aspects such as cAMP response element-binding (CREB) protein and the extracellular signal-regulated kinase cascade (Ivanov et al., 2006). Conversely, for other authors, toxic stimuli through NMDARs are uniformly characterized by an obligatory link to massive mitochondrial Ca^{2+} loading, whatever receptor location (Stanika et al., 2009). If the bases for differences between synaptic and extrasynaptic NMDAR signaling are still outstanding questions, the mechanism of excitotoxicity has been reviewed in the light of these new data.

Excitotoxicity has been described as a mechanism leading to neuronal death and was widely described in acute neuronal injuries such as trauma and stroke (Choi et al., 1988; Rothman and Olney, 1995). However, it is also an important feature of neurodegenerative diseases. For example, it was suggested that excessive NMDAR activity contributes to the pathogenesis of Huntington's disease, a disorder characterized by degeneration of striatal medium-sized spiny neurons. It is worth noting that NMDAR-dependent plasticity and transmission are altered in Huntington's disease transgenic mice much before the onset of motor deficits. Two important recent papers provided strong arguments suggesting that the balance between synaptic and extrasynaptic NMDAR activity could be crucial in determining neuronal cell survival in Huntington's disease (Okamoto et al., 2009; Milnerwood et al., 2010). These studies were performed on YAC128 transgenic mice expressing mutant full-length human huntingtin (mtHTT) protein that contains a 128-length polyglutamine expansion. Data clearly indicate increased NR2B-containing extrasynaptic NMDARs in the striatum of transgenic mice. Therefore, augmented extrasynaptic NMDAR signaling is detected at ages preceding motor dysfunction and neuronal loss. As mentioned before, extrasynaptic NMDAR activity is coupled to a CREB-inactivating dephosphorylation signal. Consistent with this, CREB phosphorylation levels are reduced in the striatum of YAC128 mice.

The fact that a direct link was revealed between sustained NMDAR activation and neuronal Aβ production (see above) led us to investigate the relative part of the two pools of NMDARs in this synthesis. Thus, we recently studied the influence of a selective stimulation of both populations of receptors on APP metabolism and Aβ production (Bordji et al., 2010). In primary cultures of cortical neurons, prolonged stimulation of extrasynaptic, but not synaptic, NMDARs importantly increased the neuronal production of Aβ. This effect was preceded by a shift from APP695 (the neuronal isoform of APP) to KPI-APPs, isoforms exhibiting an important amyloidogenic potential. By contrast, no induction of KPI-APP expression and no increase in neuronal Aβ synthesis were observed after synaptic NMDAR activation. Interestingly, intracellular calcium concentration measured after extrasynaptic NMDAR activation was lower than after synaptic activation. Thus, the modifications observed on APP and Aβ metabolism cannot be explained by a higher calcium entry but rather suggest distinct signaling pathways for each pool of receptors. Indeed, changes in neuronal APP expression pattern mediated by extrasynaptic NMDAR activation was regulated at an alternative splicing level involving calcium/calmodulin-dependent protein kinase IV. A major finding of this study is that a deregulation of the glutamatergic neurotransmission involving extrasynaptic NMDARs increases levels of neuronal Aβ synthesis. Thus, this could represent a causal risk for developing AD by promoting accumulation of this pathological protein, one of the principal hallmarks of the disease.

Taken together, these data demonstrate that NMDAR overstimulation is a common feature of various acute and chronic neurological disorders such as AD. By contrast, this subtype of glutamate receptors is known to mediate important physiological neural functions. They also indicate that, in addition to the stimulus intensity that will determine the level of neuronal calcium influx, the location of the NMDARs is a crucial parameter for determining neuronal consequences. Thus, to avoid unwanted adverse side effects, the ideal therapy would selectively block excessive NMDAR activity without affecting its physiological and neuroprotective roles. Therefore, one promising strategy is to selectively target extrasynaptic NMDAR signaling while sparing synaptic signaling.

Targeting extrasynaptic NMDARs to lower Aβ peptide: interest of memantine

For many years, excitotoxicity, and especially the NMDAR contribution, has represented a particularly attractive therapeutic target because it is implicated in the pathophysiology of a wide variety of acute and chronic neurodegenerative disorders. However, the unique relation between NMDAR overactivation and neuronal death is challenged by their parallel role in neuronal plasticity and neurotrophic processes (Hardingham, 2006; Hetman and Kharebava, 2006). Thus, to date, NMDAR antagonists that showed promise as inhibitors of excitotoxicity also blocked normal synaptic functions. Consequently, they had severe and unacceptable side effects explaining the failure of these drugs in clinical trials (Ikonomidou and Turski, 2002). However, one molecule, identified in the early 1990s as a NMDAR antagonist, was shown to be effective for the treatment of AD with an excellent clinical safety profile: memantine (Chen et al., 1992). Memantine is an uncompetitive, open-channel blocker with strong voltage dependency and a relatively rapid off-rate from the channel (Lipton, 2006). To date, it is the only clinically

approved NMDAR antagonist for the treatment of moderate-to-severe forms of AD (2002 in Europe; 2003 in the USA) (Chen and Lipton, 2006; Parsons et al., 2007). Memantine was shown to slow the cognitive decline associated with AD after as little as 2 weeks of treatment (Johnson and Kotermanski, 2006). Because of the deficit in the cholinergic system in the brain of AD patients, the major alternative therapies in AD are all acetylcholinesterase inhibitors that also act on peripheral cholinergic synapses. The different properties of memantine allow the drug to limit pathological activity of the NMDARs while relatively sparing normal synaptic activity. In fact, different independent papers reported that, unlike other classical NMDAR antagonists (e.g., MK801, AP5), memantine preferentially blocks extrasynaptic over synaptic NMDAR currents in neurons (Léveillé et al., 2008; Xia et al., 2010). The importance of maintaining normal synaptic NMDA signaling has been interestingly underlined by Papadia et al. who showed that synaptic NMDA activity is necessary to boost intrinsic antioxidant defenses (Papadia et al., 2008).

Using calcium imaging analysis, we have demonstrated in primary cortical neuron cultures that memantine significantly blocked extrasynaptic NMDAR response (Léveillé et al., 2008). This induced neuroprotection against excitotoxicity triggered by NMDA bath treatment. The fact that memantine does not affect synaptic NMDAR response is in accordance with previous results showing that memantine only acts under pathological activation of NMDARs, without affecting normal synaptic neurotransmission (Lipton, 2007). This pharmacological feature could explain the favorable safety profile of this compound in its clinical use in humans.

The Lipton group first reported that memantine binds at or near the Mg^{2+} site in the NMDAR-associated channel and they recently provided evidence explaining the discriminative action of memantine (Xia et al., 2010). Under normal conditions, at resting membrane potentials, most NMDARs are blocked by extracellular Mg^{2+}, which occupies the channel. Conversely, under pathological conditions, the ion channel is continuously open and Mg^{2+} block is mostly relieved. Therefore, memantine at low doses (up to 10 μM) predominantly acts as an open-channel blocker in the presence of prolonged elevation of glutamate concentration, as seen for extrasynaptic receptors, but is relatively inactive when glutamate is elevated for only milliseconds, as in synaptic transmission.

The efficacy of memantine to antagonize neurodegenerative pathways has been elegantly demonstrated in the context of Hungtinton's disease pathology in recent *in vivo* studies (Okamoto et al., 2009; Milnerwood et al., 2010). Given the link evidenced between NMDAR dysregulation and AD (see above), it seemed pertinent to evaluate the capacity of memantine to modulate Aβ release in *in vitro* and/or *in vivo* models. In fact, memantine has been previously shown to protect hippocampal cells from the neurotoxic effects of $A\beta_{1-40}$ in rats and to improve spatial learning in transgenic mice overexpressing mutated human APP and PS1 (Scholtzova et al., 2008; Song et al., 2008). However, scarce data are available showing that memantine could modulate the production of Aβ. A study recently reported that memantine alone, at therapeutically relevant concentrations (1–4 μM), significantly lowered the levels of sAPP and $A\beta_{1-40}$ in human neuroblastoma cells (SK-N-SH) (Alley et al., 2010). Because this undifferentiated cell line has been shown to express no detectable NMDAR activity, it was therefore concluded that the mechanism by which memantine acts on SK-N-SH cells is likely to be NMDAR independent. Two days treatment of primary rat cortical neuronal cultures with the same concentrations of memantine led to a decrease in the potentially amyloidogenic $A\beta_{1-42}$ secretion. Here again, the age of the culture (7 days) was not sufficient for neurons to express functional NMDARs. Therefore, the observed reduction of $A\beta_{1-42}$ by memantine within 2 days post-treatment in primary cultures suggests a non-NMDAR-mediated mechanism. In APP/PS1 transgenic mice exhibiting high brain levels of $A\beta_{1-42}$, oral administration of memantine (20 mg/kg/day for 8 days) produced a plasma drug concentration of 0.96 μM and significantly reduced the cortical levels of soluble $A\beta_{1-42}$. The ratio of $A\beta_{1-40}/A\beta_{1-42}$ also increased in treated mice, suggesting a combination of mechanisms that could bypass the NMDAR and act on the γ-secretase complex. In complement to these findings, long-term treatments (4 months) of a different APP/PS1 transgenic mouse model with memantine (10 mg/kg/day; intraperitoneal) have shown significant reduction of amyloid plaques in these animals. This suggests that memantine can rapidly reduce Aβ production, although longer term treatments are required to alter insoluble Aβ plaques.

In a recent study, we have evidenced a direct relationship between prolonged extrasynaptic NMDAR activation and neuronal Aβ production (Bordji et al., 2010). The release of Aβ was preceded by the neuronal induction of KPI-APPs, isoforms exhibiting an important amyloidogenic potential. Taking into account previous data of our group, a mechanism was therefore proposed to establish the link between an overproduction of Aβ and a deregulation of the glutamatergic neurotransmission involving NMDAR activation (Figure 1). We further showed that memantine dose dependently inhibited extrasynaptic NMDAR-induced KPI-APP protein expression as well as neuronal $A\beta_{1-42}$ release. Interestingly, these effects were observed at a clinically achievable concentration (1 μM), after a prolonged time of activation (24 h), thus reflecting a pathophysiological situation. This is in accordance with previous observations reporting that memantine only acts under pathological conditions without much affecting normal functions, and thus relatively spares normal excitatory synaptic activity (Lipton, 2007). Because of the impossibility to adapt *in vivo* a protocol of extrasynaptic NMDAR activation, these *in vitro* data were partially confirmed in animal studies. NMDA intraperitoneal injections increased cortical KPI-APP expression in mice, whereas low-dose memantine, described to block extrasynaptic NMDAR, significantly inhibited KPI-APP induction.

Conclusion

Calcium ions play crucial roles in neuronal cell functions such as synaptic plasticity, membrane excitability and gene expression. If tau and Aβ are identified as the causative factors for

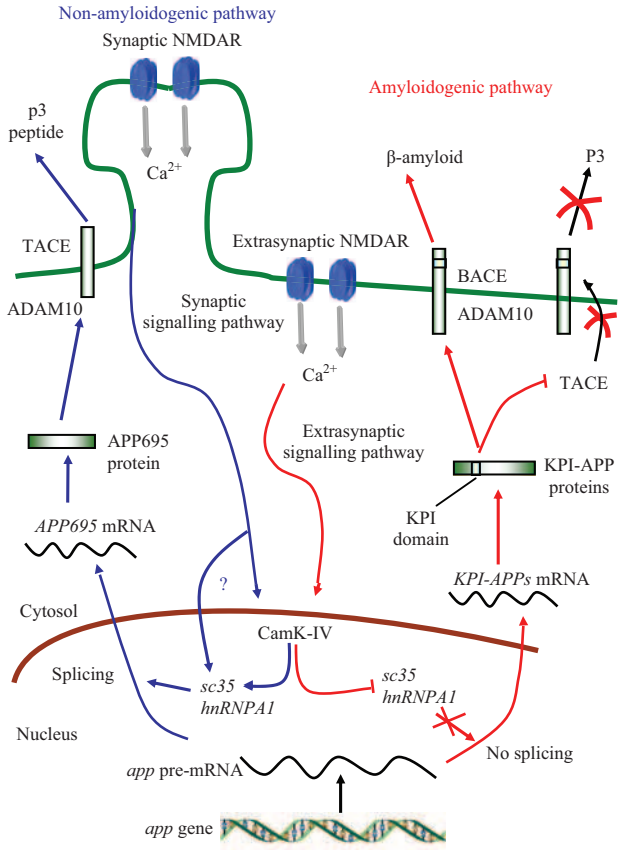

Figure 1 Extrasynaptic NMDAR activation is selectively involved in neuronal Aβ release.

Neuronal calcium entry through extrasynaptic NMDARs activates selective calcium signaling pathways (still unknown) leading to activation of intranuclear CaMK IV. CaMK IV subsequently represses pre-mRNA splicing of several neuronal transcripts of proteins such as ion channel receptors or transmembrane proteins (APP). In the case of APP, CaMK IV inhibits skipping of exon 7 (corresponding to KPI domain). This inhibition could be through the downregulation of two splicing factors: heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and SC35. Subsequent retention of exon 7 leads to an increase in neuronal KPI-APP expression, an isoform which is normally weakly expressed in neurons. The KPI domain associates with the α-secretase candidate tumor necrosis factor-α converting enzyme (TACE), leading to inhibition of its enzymatic activity. The consequence of this KPI-dependent inhibition is a shift from α-secretase to β-secretase-mediated APP processing leading to an increase in Aβ production. Such a mechanism has not been evidenced after synaptic NMDAR activation. By contrast, normal splicing events on APP pre-mRNA were maintained and no induction of KPI-APP mRNA or protein expression in neurons was detected. Thus, in neuronal culture models, no increase in Aβ secretion was measured after activation of the synaptic pool of NMDARs. According to this model, the two populations of NMDARs activate two distinct calcium signaling pathways, one of (extrasynaptic) modifying APP expression pattern, finally leading to neuronal Aβ overproduction. Memantine was shown to counteract this process by preferentially targeting extrasynaptic NMDARs, confirming the interest of this NMDAR antagonist in the treatment of AD.

the disease, there is accumulating evidence pointing to the importance of disturbed calcium signaling in AD pathogenesis. Aging, especially linked to the development of AD, also involves significant changes in calcium homeostasis, which could contribute to the initiation of the disease. Both fibrillar Aβ and Aβ oligomers were shown to have deleterious effects on synaptic function and neuronal fate in the context of AD. Thus, accumulation of Aβ outside the cell but also intracellularly, within synapses, is a major event of AD pathogenesis. Several studies have provided evidence that synaptic activity modulates Aβ production and calcium entry through NMDARs was identified as a major component in the control of neuronal Aβ synthesis. Very recently, the extrasynaptic pool of NMDARs has been identified to be involved in the pathogenesis of neurodegenerative diseases. In AD, prolonged extrasynaptic NMDAR activation induces increased neuronal Aβ release. Memantine, a well-tolerated drug that has already been approved for clinical use, was shown to preferentially target extrasynaptic NMDARs and to lower Aβ synthesis. Thus, targeting the extrasynaptic pool of receptors could importantly contribute to the clinical efficiency and tolerability of memantine. However, very few studies reporting such effects in the field of AD have been published. Other mechanisms, perhaps NMDAR-independent and that could affect β- and/or γ-secretase activities, are not excluded. Therefore, further studies need to be performed to investigate the pathogenic pathways that could link NMDAR dysregulation and Aβ overexpression and the precise mechanism of action of memantine.

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